TOBACCO INDUSTRY RESEARCH COMMITTEE

CONFIDENTIAL

TIRC Grant #170

Report No. 1

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September, 1958

A Study of Structure-Activity Relationships Among Drugs Which Affect Nicotine-Sensitive Physiological Mechanisms

Experiments were performed to determine the effects of nicotine, and several other agents which block ganglionic transmission, on the responses of the cat nictitating membrane to stimulation of the preganglionic nerve fibers at both low and high rates of stimulation.

Methods: Experiments were performed on 25 adult cats of both sexes, which had been anesthetized with sodium pentobarbital (35 mg./K., intraperitoneally). In each experiment, the trachea was cannulated, and the animal breathed spontaneously throughout the experiment. Femoral blood pressure was recorded kymographically with a mercury manometer; intravenous injections were made via a femoral vein catheter. A stainless steel cannula was passed retrograde into the right lingual artery; drug injections through the cannula could be directed toward the head or toward the ipsilateral superior cervical ganglion by occluding the common carotid artery below or above, respectively, the origin of the lingual artery. Square wave stimuli of 1.0 msec. duration and of supramaximal voltage were applied from a Grass S4C stimulator via shielded platinum electrodes to the cephalic end of the cut preganglionic cervical sympathetic trunk on the right side. Contractions of the right nictitating membrane were recorded kymographically by means of an isotonic lever system of 7-fold magnification; tension on the nictitating membrane was 3.0 Gm.

In each experiment, trains of stimuli were applied to the preganglionic fibers for periods of 20 seconds every 2 to 3 minutes, specifically, as soon after a train of stimuli had been given as the length of the nictitating membrane returned to a steady level. During the first 10 seconds of each period of stimulation a frequency of stimulation of 1.25 stimuli/second was used; during the second half of each period a frequency of 20 stimuli/second was used.

For each train of stimuli the maximum degree of contraction of the nictitating membrane during the periods of low and high frequency stimulation, respectively, were determined from the kymograph record by measuring the maximum deviation of the writing lever, during the periods of low and high frequency stimulation, from the base-line record of nictitating membrane length recorded before the onset of the train of stimuli. The degree of contraction of the membrane at the end of the period of high frequency stimulation ("high frequency response";HFR) was always greater, under control conditions, than the degree of contraction at the end of the period of low frequency stimulation ("low frequency response";LFR). In control experiments it was determined that the same HFR was produced whether or not an LFR preceded it.

In each experiment trains of stimuli were delivered to the preganglionic fibers at intervals, as described above, until constant HFR's and LFR's were produced. Then a preselected dose of one of the drugs under investigation was administered to the ganglion via the lingual artery cannula. One minute after the end of the injection, a train of stimuli was applied to the nerve, and trains of stimuli were then applied successively as described above. When the HFR and LFR were judged to have returned to a stable level, a

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second drug dose was given, and the sequence of trains of stimuli was repeated. Care was taken to avoid interaction of the effects of successive drug doses. Most of the cats studied received more than one drug.

The drugs which have been studied thus far are nicotine, tetraethylammonium bromide (TEA), hexamethonium chloride (C6), and morphine sulfate. The drugs were prepared as stock solutions in 0.9% sodium chloride solution, and the pH's of the solutions were adjusted to 7.4 with hydrochloric acid and sodium hydroxide. Dilutions have been prepared from stock solutions, and buffered to pH 7.4, as they have been needed. Drug solutions were refrigerated between periods of use, but were allowed to reach room temperature before being used. Control injections of buffered 0.9% sodium chloride solution, at room temperature, have consistently failed to alter either the HFR or LFR.

Results: All of the drugs which were tested produced at some dose level a decrease in the response of the nictitating membrane to preganglionic stimulation. Data in the literature indicate that failure of ganglionic transmission, rather than failure of nerve conduction, neuro-effector transmission, or effector activity is responsible for this decreased response in the case of all of the agents we have studied. With effective doses of C6 (20-120 mcgm., intraarterially*), TEA (200-1000 mcgm., i.a.) and morphine (100-400 mcgm., i.a.) the only effects observed were decreases in the response of the nictitating membrane; contraction of the membrane has not been produced by any of these agents. Nicotine, in doses larger than 2-3 micrograms, invariably produced contraction of the membrane when the dose was directed toward the ganglion, but never produced contraction when the injected mass was directed toward the head and membrane itself. In general, the magnitude of the contraction produced by nicotine was proportional to the dose of nicotine used; maximal contractions were produced with doses of 20-80 micrograms. Low doses of nicotine (10-15 mcgm. or less, i.a.) occasionally facilitated the HFR's and LFR's invoked immediately after relaxation of the membrane after the nicotine-induced contraction. Larger doses of nicotine (20-80 mcgm., i.a.) regularly caused a decreased response of the membrane to preganglionic stimulation. Both the facilitatory and inhibitory effects of nicotine on the HFR and LFR were, in general, proportional to the dose which produced them.

All of the drug effects which we have observed were completely reversible. With all of the drugs studied, characteristic effects on HFR and LFR were observed after doses which produced no alteration in systemic blood pressure. With the larger doses, particularly of TEA and nicotine, alteration in the response of the membrane was frequently accompanied in time by changes of blood pressure such as are usually produced by smaller doses of the drugs given intravenously.

With regard to their ganglionic "blocking" effects, nicotine, TEA, and morphine and C6 differed in their effects on HFR and LFR, as determined at the times of maximal drug effect. Nicotine, TEA, and morphine depressed the LFR relatively more than they did the HFR. In contrast, as Paton and Zaimis observed, C6 depressed the HFR relatively more than the LFR. These differential effects of the drugs can be demonstrated by comparing, for the different drugs, the change produced in HFR (HFR at time of maximal effect/HFR before drug administration; HFR_a/HFR_b) with the change produced in the corresponding LFR (LFR at time of maximal effect/LFR before drug administration; LFR_a/LFR_b). A

* Dose ranges include the minimally and maximally effective doses studied in all experiments.

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demonstration of this kind, of results obtained after all doses, in all experiments with nicotine, TEA and C6 is given in the table:

DRUG	No. of Cats	No. of Injections	No. of Effective Injections*	INCIDENCE OF RESPONSES $HFR_a/HFR_b > LFR_a/LFR_b$	$HFR_a/HFR_b = LFR_a/LFR_b$	$HFR_a/HFR_b < LFR_a/LFR_b$
Nicotine	8	45	30	21	2	7
TEA	10	52	51	42	0	9
Morphine	2	4	4	4	0	0
C6	7	29	29	7	0	22

* Injections were considered to be effective when the LFR was decreased by the drug.

Comparison of the tabulated pattern of responses for nicotine and C6 and for TEA and C6 by the method of Chi-square indicated that the patterns for nicotine and C6 (Chi-square = 12.7) and TEA and C6 (Chi-square = 21.7) would occur much less than once in a hundred times on the basis of random sampling from a single population of responses. In contrast, comparing the pattern of responses for nicotine and TEA indicated (Chi-square = 0.24) that such a pattern would be observed at least six times out of ten if responses were drawn randomly from a single population. We infer that nicotine and TEA produce qualitatively similar patterns of alteration in HFR and LFR, which differ from that produced by C6, and that nicotine and TEA may have similar mechanisms of action on ganglionic transmission which may differ from that of C6. Too few experiments have been performed with morphine to permit meaningful analysis by the method of Chi-square. Data obtained with morphine indicate its pattern of responses is similar to that produced by nicotine and TEA.

Rosenblueth demonstrated that partial transection of preganglionic fibers to the superior cervical ganglion distal to a stimulating electrode (i.e., decreasing the quantity of neurotransmitter released for each stimulus without decreasing the amount of transmitter released per neurone activated) caused a greater decrease in the LFR of the nictitating membrane than in the HFR. Paton, and Schaumann, concluded that morphine (which in our preparation affects LFR more than HFR) caused a decrease in the release of acetylcholine (ACh) from the myenteric plexus of the guinea pig. Paton and Zaimis observed that C6 blocks ganglionic transmission and the response to exogenous ACh without altering the release of ACh from the ganglion, and that C6 affects the HFR more than the LFR. Paton and Zaimis concluded that C6 acts at the ganglion by decreasing the number of receptors on the post-synaptic cell bodies available to ACh released in normal amounts from the pre-synaptic nerve terminals. Considering our results with nicotine, TEA, and morphine in the light of these observations lead us to the hypothesis that nicotine, TEA, and morphine might decrease ganglionic transmission by decreasing the release of ACh from the pre-synaptic nerve terminals, and that the action of these compounds might differ qualitatively from those of C6, which we believe acts in the manner suggested by Paton and Zaimis.

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To test quantitatively the degree to which our data fitted this hypothesis, the following procedure was carried out:

1. We determined that with our recording system, magnitude of contraction of the nictitating membrane was linearly related to the logarithm of the frequency of supramaximal stimuli applied to the preganglionic fibers. The curve passed through the points: (20 stimuli/second, 100% response), (2.5 stimuli/second, 50% response).

2. The following assumptions were made:

a. The amount of ACh released from pre-synaptic nerve terminals per unit time is a linear function of stimulus frequency; ACh release at a frequency of 20 stimuli/second was arbitrarily considered to equal 100 Units, and at a frequency of 1.25 stimuli/second, to be 6.25 Units.

b. The per cent of the total available receptors on the post-synaptic cell bodies occupied by ACh is a logarithmic function of the ratio: (ACh Units released per unit time/total number of receptors available).

c. The magnitude of contraction of the nictitating membrane is a linear function of the per cent of the available receptors occupied by ACh.

d. Drugs may affect ganglionic transmission either by decreasing the release of ACh or by making receptors unavailable to ACh, or by both mechanisms. (We recognize, but have not included in our theoretical analysis, the possibility of influencing ganglionic transmission by other means too, for example, by altering the rate of hydrolysis of ACh.)

3. Computations were made which yielded theoretical magnitudes of HFR and LFR under control conditions and when hypothetical decreases of varying amounts were made in ACh output per nerve volley or in the number of post-synaptic receptors available to ACh.

4. Curves were plotted, each of which showed the theoretical relationship between HFR and LFR before and after the administration of drugs which decreased ganglionic transmission by one of the two mechanisms hypothecated in "d." above. On these curves, the abscissal unit was LFR_a/LFR_b, and the ordinal unit was $(HFR_a/LFR_a) / (HFR_b/LFR_b)$. These units are dimensionless and permit combining all the data obtained with a given drug in one or more experiments on a single graph. The metameter of dose is eliminated from data plotted in this way. The theoretical curves are presented in Figure 1.

5. For each drug studied, the ratios described in "4." above were computed for responses to all stimulus trains, for all drug doses in all experiments. It should be noted that ratios were computed for all responses elicited during the entire course of action of each drug dose. The experimental data were plotted as were the theoretical ratios described above. Typical data are presented in Figures 2, 3, and 4.

It can be seen by inspection of Figures 2 and 3 that data obtained from experiments with nicotine and TEA fit closely to the theoretical curves obtained using the assumption that decreased ganglionic transmission was effected only by

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decreasing ACh output from pre-synaptic nerve terminals. Inspection of Figure 4, on the other hand, indicates that data obtained from experiments with C6 fit the theoretical curve plotted using the assumption that decreased ganglionic transmission was produced only by decreasing the number of receptors available to ACh released in normal quantities from the pre-synaptic nerve terminals. However, it is obvious that more data must be collected before the precise fit of data for C6 to the theoretical curve can be determined.

Conclusions: Our data are consistent with our hypothesis that nicotine, TEA, and morphine, under the conditions of our experiments, decrease transmission through the superior cervical ganglion of the cat by interfering with the release of ACh from pre-ganglionic nerve terminals. Our data support the view that the ganglionic effect of C6 is produced by making receptors on the post-synaptic cell body unavailable to ACh released in normal quantities.

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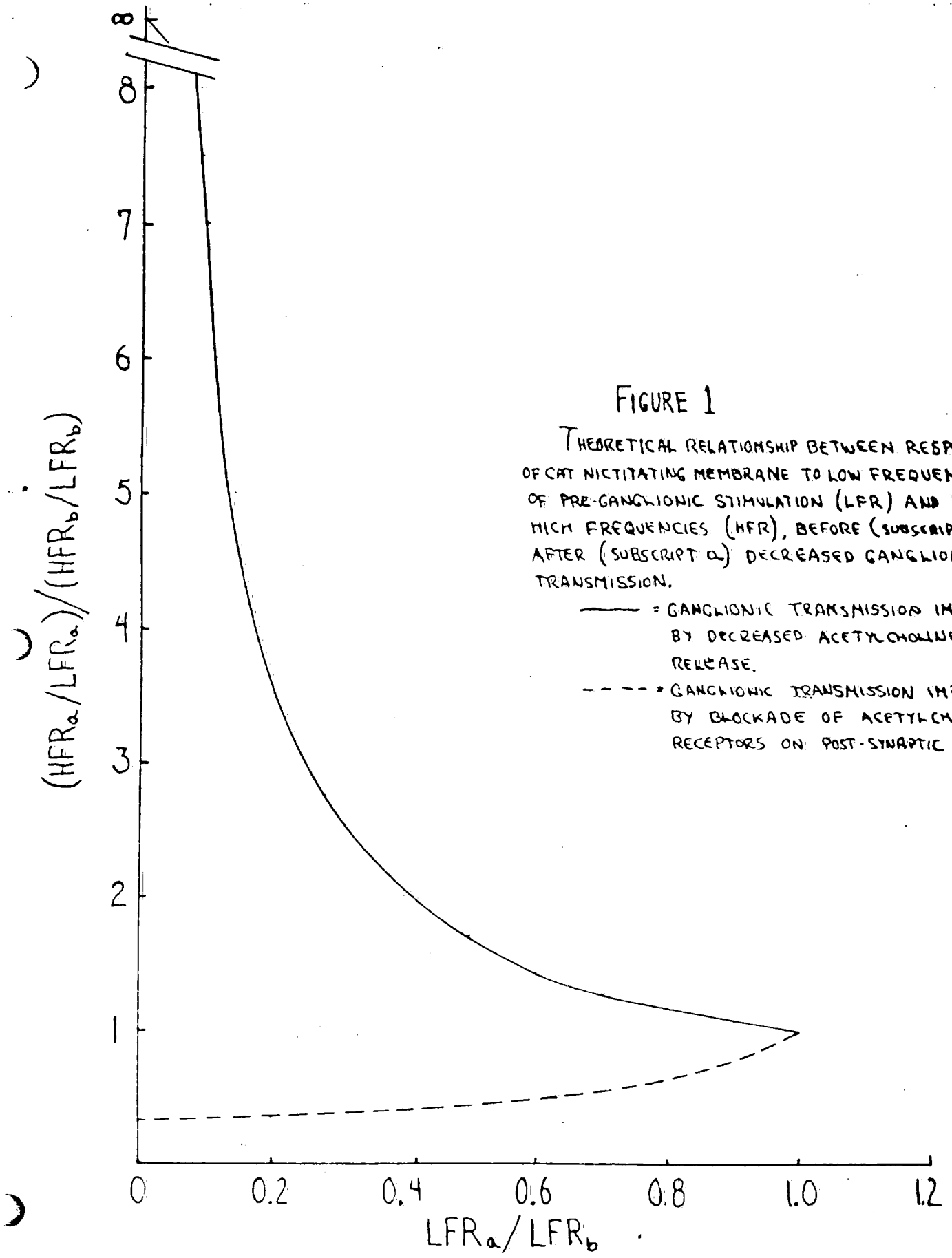

FIGURE 1

THEORETICAL RELATIONSHIP BETWEEN RESPONSE OF CAT NICTITATING MEMBRANE TO LOW FREQUENCIES OF PRE-GANGLIONIC STIMULATION (LFR) AND TO HIGH FREQUENCIES (HFR), BEFORE (SUBSCRIPT b) AND AFTER (SUBSCRIPT a) DECREASED GANGLIONIC TRANSMISSION.

——— = GANGLIONIC TRANSMISSION IMPAIRED BY DECREASED ACETYLCHOLINE RELEASE.

- - - - = GANGLIONIC TRANSMISSION IMPAIRED BY BLOCKADE OF ACETYLCHOLINE RECEPTORS ON POST-SYNAPTIC CELL

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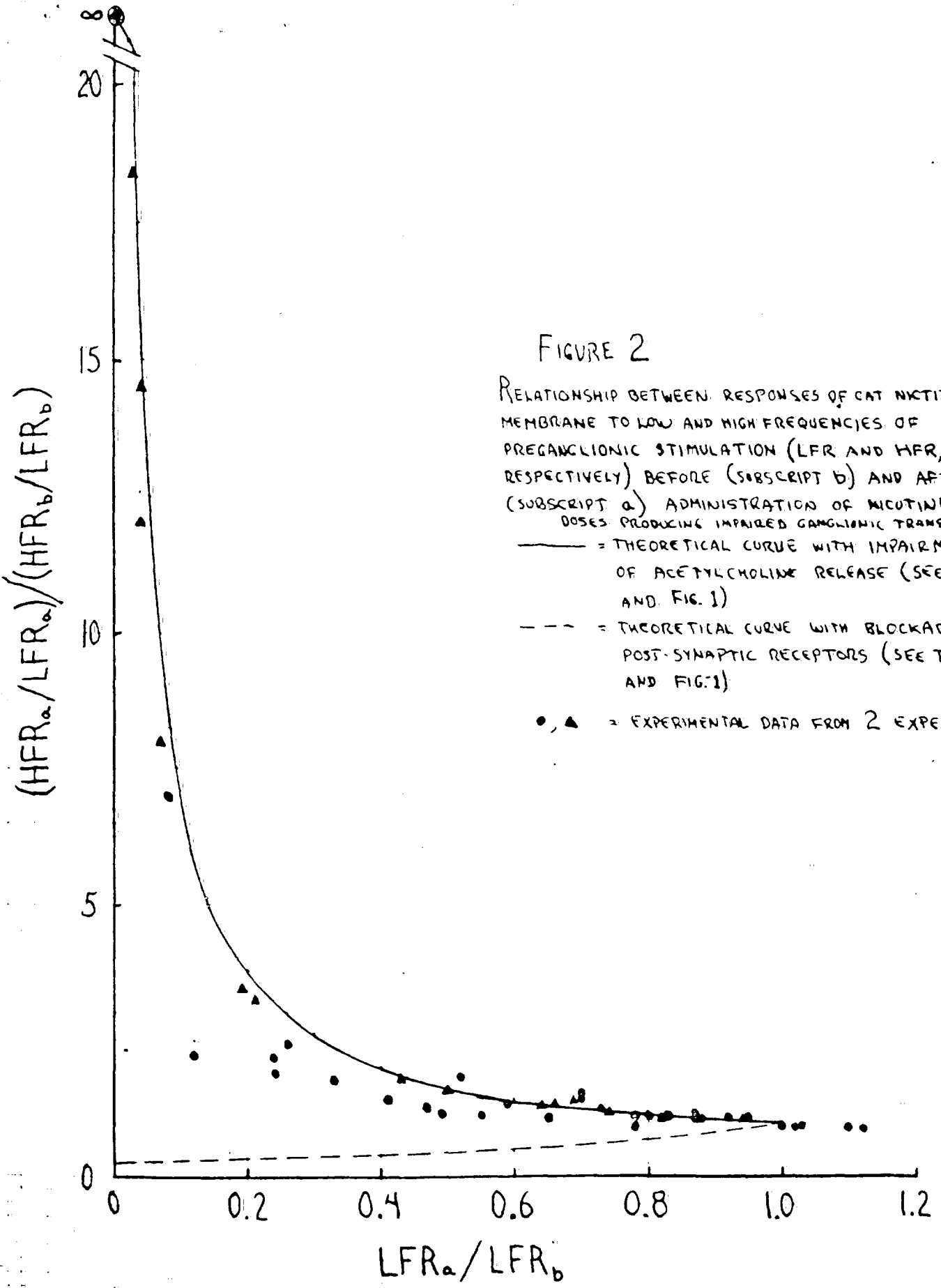

FIGURE 2

RELATIONSHIP BETWEEN RESPONSES OF CAT NICTITATING MEMBRANE TO LOW AND HIGH FREQUENCIES OF PREGANGLIONIC STIMULATION (LFR AND HFR, RESPECTIVELY) BEFORE (SUBSCRIPT b) AND AFTER (SUBSCRIPT a) ADMINISTRATION OF NICOTINE, IN DOSES PRODUCING IMPAIRED GANGLIONIC TRANSMISSION.

——— = THEORETICAL CURVE WITH IMPAIRMENT OF ACETYLCHOLINE RELEASE (SEE TEXT AND FIG. 1)

– – – = THEORETICAL CURVE WITH BLOCKADE OF POST-SYNAPTIC RECEPTORS (SEE TEXT AND FIG. 1)

●, ▲ = EXPERIMENTAL DATA FROM 2 EXPERIMENTS

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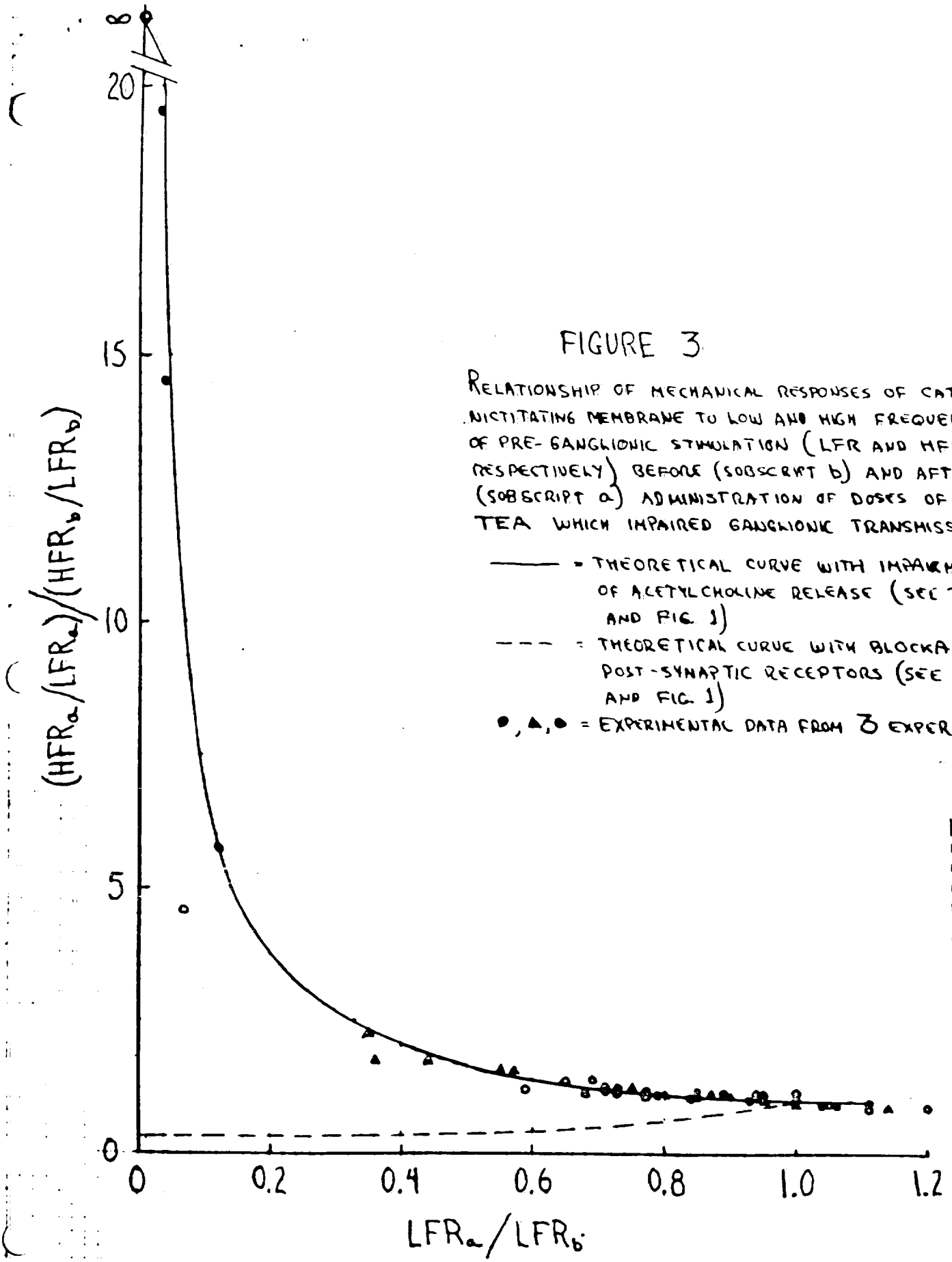

FIGURE 3

RELATIONSHIP OF MECHANICAL RESPONSES OF CAT NICTITATING MEMBRANE TO LOW AND HIGH FREQUENCIES OF PRE-GANGLIONIC STIMULATION (LFR AND HFR, RESPECTIVELY) BEFORE (SUBSCRIPT b) AND AFTER (SUBSCRIPT a) ADMINISTRATION OF DOSES OF TEA WHICH IMPAIRED GANGLIONIC TRANSMISSION.

——— = THEORETICAL CURVE WITH IMPAIRMENT OF ACETYLCHOLINE RELEASE (SEE TEXT AND FIG. 1)

– – – = THEORETICAL CURVE WITH BLOCKADE OF POST-SYNAPTIC RECEPTORS (SEE TEXT AND FIG. 1)

●, ▲, ● = EXPERIMENTAL DATA FROM 3 EXPERIMENTS

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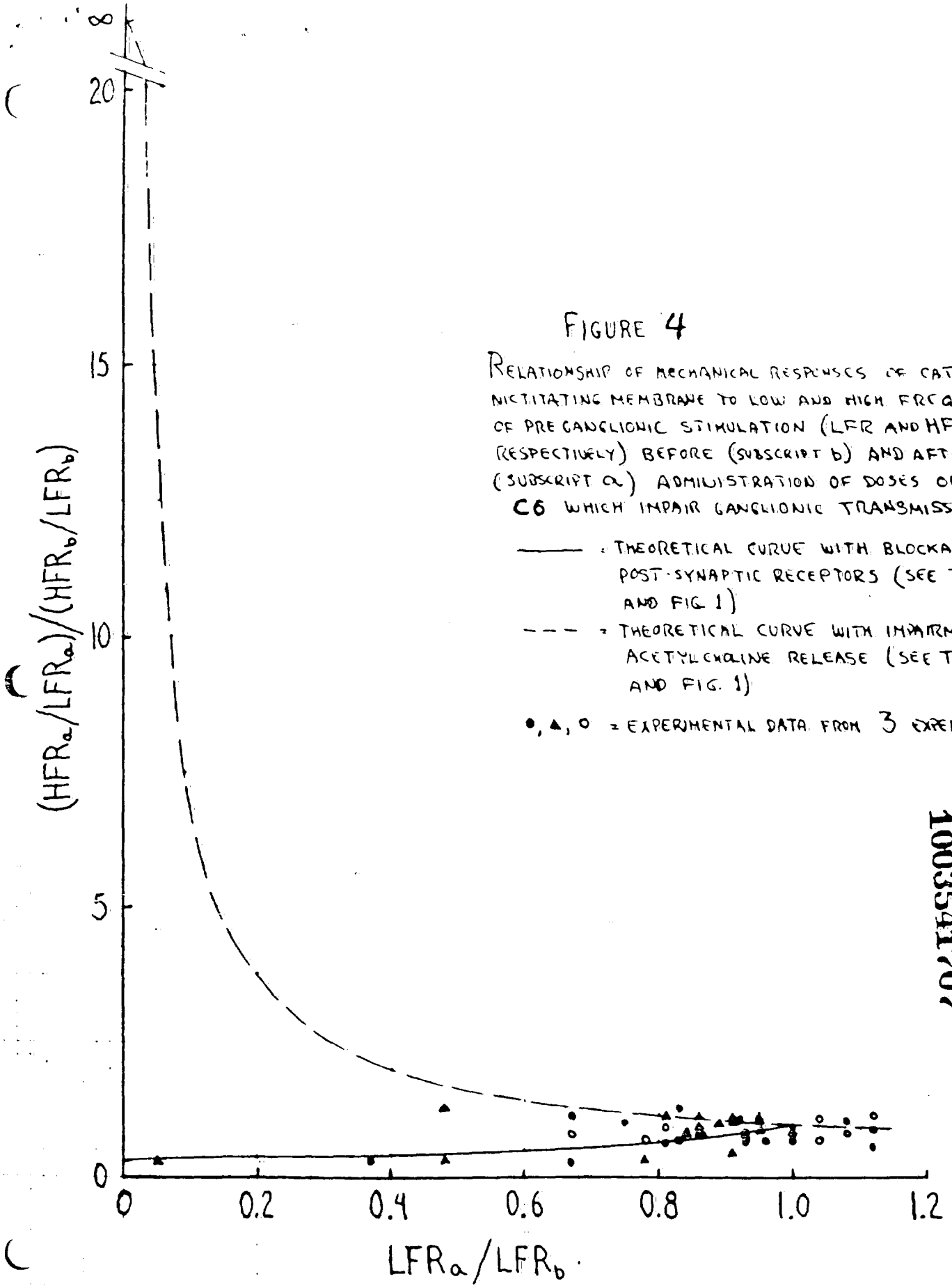

FIGURE 4

RELATIONSHIP OF MECHANICAL RESPONSES OF CAT NICTITATING MEMBRANE TO LOW AND HIGH FREQUENCIES OF PREGANGLIONIC STIMULATION (LFR AND HFR, RESPECTIVELY) BEFORE (SUBSCRIPT b) AND AFTER (SUBSCRIPT a) ADMINISTRATION OF DOSES OF C6 WHICH IMPAIR GANGLIONIC TRANSMISSION.

——— = THEORETICAL CURVE WITH BLOCKADE OF POST-SYNAPTIC RECEPTORS (SEE TEXT AND FIG. 1)

– – – = THEORETICAL CURVE WITH IMPAIRMENT OF ACETYLCHOLINE RELEASE (SEE TEXT AND FIG. 1)

•, ▲, ○ = EXPERIMENTAL DATA FROM 3 EXPERIMENTS

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